catalyst structure–function relationships in a visual manner at the microscopic level.

The catalyst bodies chosen in this current study were typical of those used in industrial settings — here an active catalyst, microporous zeolite ZSM-5, was dispersed in a macroporous clay matrix, which acted as binder, rendering the resulting material stronger and more stable. The resulting catalysts exhibit a complex hierarchical pore arrangement arising from each component's pores as well as their inter- and intra-connectivity. The pores' architectures were visualized by staining with fluorescent nanoprobes of various sizes. In a typical experiment, cylinder-shaped extrudates of the catalyst with a diameter of 1, 2 or 3 mm were incubated with a solution containing the fluorescent nanoprobe. In this experiment, the smallest nanoprobe *N,N′*-bis-(2,6-dimethylphenyl)-perylene-3,4,9,10-tetracarboxylic diimide (PDI), was chosen as it has a molecular structure and size comparable to the catalyst reaction products, the PAHs, and represented a 2-nm probe (Fig. 1); larger 20-, 45- and 100-nm nanoprobes were also used.

The ZSM-5 catalyst was incubated with a nanoprobe for 24 hours, to enable nanoprobe uptake and retention, then dried and bi-sectioned. Confocal fluorescence microscopy was then used to evaluate its pore architecture. While the penetration of the nanoprobes into the catalyst pores was highly dependent on the incubation time and nanoprobe size, fluorescence staining often revealed a penetration boundary, shown in Fig. 1, indicating a significant pore narrowing, and/or a disconnection of the pore networks, immediately after that boundary. These results underscore the value of visualization when comparing them with the averaged pore distribution results determined by conventional techniques. Porosimetry measurements of the same ZSM-5 extrudates revealed the existence of large pores, of averaged diameter 600 nm, when using Hg atoms as probes. Staining results, however, indicated that the core regions of the 2- and 3-mm bodies were not accessible by smallest nanoprobe — the 2-nm PDI species— from the exterior.

In order to correlate the porous structure of catalysts with their catalytic performance, the molecular transport of PAHs was subsequently investigated by fluorescence microscopy. It is generally believed that PAH products are involved in the deactivation of catalysts as they tend to block active sites and stick in the channels by forming cokes — a notion that is supported by the present study. Alternative excitation with 488-nm and 642-nm lasers led to green and red emissions, corresponding to small (less conjugated) and large (more conjugated) PAH species, respectively. It was found that, in the early stage of the MTH reaction (under 15 minutes), smaller (green) and larger (orange) PAHs were produced in the shell and core regions of the catalyst, respectively. When the reaction time was extended to 75 minutes, the shell layer was completely occupied by non-fluorescent cokes and an emission colour change from green to black was observed. At the same time, the penetration boundary set a barrier that prevented the formed PAHs in the core region from exiting. These PAHs then underwent oligomerization and intramolecular transformation to produce larger molecular species. As shown in Fig. 1 (right), one can clearly identify a consistency between the structural boundary (from incubation with external nanoprobes, top half) and activity boundary (from MTH reaction products, bottom half), indicating the structural basis of the catalytic activity.

This study benefits the single-particle catalysis and chemical imaging fields by establishing an approach to visualizing the accessibility of pores within single catalyst particles, which is more relevant to the chemical activity of the catalyst than the conventional measures of average porosity of the bulk material. Although millimetre-sized catalyst extrudates were studied in this work, similar strategies may be applicable to variously sized micro-crystals and nanomaterials such as metal–organic frameworks, nanotubes and nanochannels, by combining this approach with super-resolution microscopy[8]. Furthermore, single-entity chemistry has in the past often focused on microscopic studies by using model reactions and isolated catalysts[4,5,8]. While these are powerful tools for mechanistic studies, stronger connections between single-entity studies and ensemble performance in more practical systems are required to better understand the structure and activity of catalysts, in particular how the architecture and boundaries of pores affect product formation and catalyst deactivation. These insights may help better inform the study and design of catalysts. ❐

Wei Wang
State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, China.
e-mail: wei.wang@nju.edu.cn

Published online: 5 November 2018
https://doi.org/10.1038/s41557-018-0168-7

References
1. Fredrich, J. T., Menendez, B. & Wong, T. F. *Science* **268**, 276–279 (1995).
2. Sambur, J. B. et al. *Nature* **530**, 77–80 (2016).
3. Hendriks, F. C. et al. *Angew. Chem. Int. Ed.* **57**, 257–261 (2018).
4. Chen, T. et al. *Chem. Rev.* **117**, 7510–7537 (2017).
5. Wang, W. *Chem. Soc. Rev.* **47**, 2485–2508 (2018).
6. Whiting, G. T., Nikolopoulos, N., Nikolopoulos, I., Dutta Chowdhury, A. & Weckhuysen, B. M. *Nature Chem.* https://doi.org/10.1038/s41557-018-0163-z (2018).
7. Olsbye, U. et al. *Angew. Chem. Int. Ed.* **51**, 5810–5831 (2012).
8. Ameloot, R. et al. *Angew. Chem. Int. Ed.* **52**, 401–405 (2013).

SYNTHETIC CELLS

Talking across the membrane

One goal of synthetic biologists is to develop artificial systems to help study biological processes. Now, cell communication and differentiation have been demonstrated using spatiotemporal patterns created in artificial multicellular compartments.

Yi Li and Rebecca Schulman

The growth of an embryo from a fertilized egg requires cells to differentiate into distinct types. This differentiation process continues as development proceeds, eventually producing numerous different types of cell, tissue and organ. While each cell in a multi-celled organism is a discrete physical compartment, carefully orchestrated communication between cells is necessary to achieve such differentiation[1]. And because cell–cell communication also enables

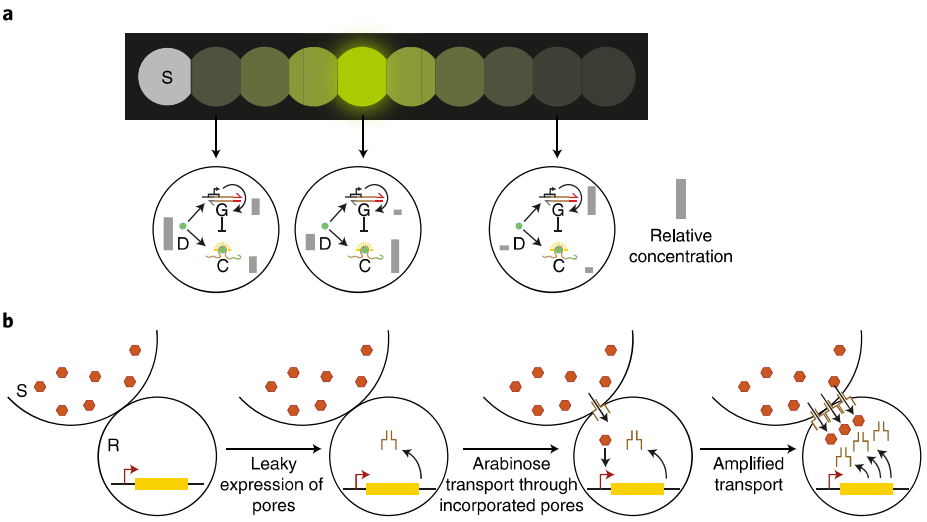

Fig. 1 | A travelling pulse and differentiation of artificial cells. a, A travelling pulse is generated along an array of artificial cells each containing a feed-forward gene network. The sender cell (S) releases signalling molecules (D) into the neighbouring receiver cell that contains a feed-forward loop circuit. These signalling molecules activate an output of green fluorescence, and subsequently activate a repressor node (G) that suppresses fluorescence output. The level of green fluorescence in each receiver cell indicates the current output level (C) of its feed-forward loop circuit triggered by these signalling molecules. **b**, Different phases of artificial cell differentiation. The α-haemolysin pores that allow transport of arabinose from the sender cell (S) into the receiver cell (R) are initially produced only because of leaky gene expression. Fluctuations in this expression can increase transport of arabinose into the cell, which can in turn trigger a positive feedback loop that amplifies transport.

cells to coordinate within structures that have already formed, it is central to the organization of cells into tissues, organs and whole organisms[2].

A key form of cell–cell communication involves two processes: the release of signalling molecules by a sender cell and the receipt of these signals by cells that subsequently respond, for example, by changing patterns of gene expression[3]. Synthetic biologists have previously shown it is possible to use such processes to engineer coordinated behaviour within communities of living cells to program bacteria to produce specific spatial patterns[4,5].

Gene circuit-like chemistries can also be synthesized for use in vitro, suggesting that including synthetic gene circuits in synthetic cells might enable synthetic cells to coordinate as living cells do — but by using artificial components to do so. Synthetic gene circuits have previously been integrated into different types of artificial cell, including lipid and polymeric vesicles, virus capsids and colloidal particles[6]. Synthetic gene circuits can also coordinate across space: spatiotemporal genetic regulation based on reaction-diffusion processes has been used to direct coordination between artificial, gene-containing compartments separated by channels within silicon chips[7]. Now, as they report in *Nature Chemistry*, Aurore Dupin and Friedrich C. Simmel have taken a key step towards creating synthetic multicellular systems[8]. They constructed a material composed of a series of artificial cells. Each artificial cell is enclosed by a lipid bilayer, which forms a separating membrane between compartments. Inside the compartments are synthetic gene circuits. Dupin and Simmel show how multiple artificial cells can coordinate via the export and import of small-molecule signals across membranes and through membrane pores. They also demonstrate how artificial cells initially containing the same circuit, or genome, might differentiate into different synthetic epigenetic states by responding to and amplifying small fluctuations in concentration that arise within them.

To create communicating multicellular networks, Dupin and Simmel used an emulsion-based technique to arrange and assemble water-in-oil droplets separated by lipid bilayers. This technique allows precise control over the designed system: each artificial cell may contain a unique type of gene circuit and may be placed at a defined location. Cell-to-cell communication may occur within this artificial network when transmembrane protein pores — α-haemolysin proteins — become incorporated in lipid bilayers, allowing signalling molecules to diffuse through the compartments. By determining the rates of transport of different small molecules across the lipid membrane and through pores, Dupin and Simmel determined how different small molecules could propagate through the network via either nonspecific membrane diffusion or pore-mediated transport. They showed how synthetic transcriptional circuits could detect and respond to two of these molecules, arabinose or *N*-hexanoyl-L-homoserine lactone (C6-HSL), by showing how the rates of expression of genes activated by either arabinose or C6-HSL differ in the presence or absence of α-haemolysin pores. The results showed that α-haemolysin pores mediate transport of arabinose, but not C6-HSL, across the membrane.

Dupin and Simmel then show how specific gene circuit dynamics can direct specific spatiotemporal patterns of fluorescence. The coupling of signal diffusion with a type-1 feed-forward loop gene circuit, which initially activates output and subsequently represses it, creates a travelling pulse of expression along a linear array of artificial cells (Fig. 1a). Within this array, each cell received from its left neighbour an activating signal that would increase the receiver cell's fluorescence by binding to an RNA aptamer in that cell. Meanwhile, upon fluorophore binding, an ssDNA activator was released that turned on transcription of a synthetic gene element, in turn releasing an element that would repress fluorophore binding. The resulting fluorescence pulse travelled along the array at an average speed of 30 μm min^{-1}; this speed could be tuned by changing initial signal concentration and transcriptional activity. The team also explored how geometry affected system dynamics by tracking such a pulse through a 2D array of 5 × 5 receiver cells. Global patterns of gene expression generated by gene networks and molecular gradients induce cell differentiation during organism development. These demonstrations of spatial pattern formation by synthetic gene circuits responding to diffusing molecules advances our understanding of how to program global synthetic gene expression patterns.

Stochastic fluctuations play an important role in biological cell differentiation[9]. Dupin and Simmel show how this differentiation strategy might be emulated in artificial cells by designing a system in which circuit noise in artificial cells might be amplified through their communication. Specifically, they constructed clusters in which four synthetic cells, each containing the same feedback circuit, surrounded a sender cell. Initially, receiver cells produced pore molecules at a

low rate, leading to relatively low amounts of flux between themselves and the sender. However, arabinose produced in the sender cell could increase pore production when transported to a receiver cell. The presence of more pores in receiver cells leads to increased arabinose influx, inducing positive feedback (Fig. 1b). The rate at which such feedback might occur should be influenced by stochastic fluctuations in pore production. Since initial pore expression levels varied between receiver cells and protein pore concentration had to reach an incorporation threshold to mediate transport, expression levels of the receiver cells should gradually diverge during such a process. Dupin and Simmel observed that receiver cells had significantly varied levels of fluorescence after four hours.

Further investigation of synthetic, coordinated multi-cell gene systems may be aided by improvements in the development of materials and the mechanics of pore-mediated communication. The emulsion method of constructing the artificial cell networks is powerful but labour intensive. Dupin and Simmel also observed that low rates of incorporation of synthetic pores limited their ability to program coupled systems in which gene circuits mediate rates of pore-assisted communication. Improvements to network construction methods and control over synthetic pores are worth investigating: coordinated chemical compartments could make it possible to integrate and allow for coordination of numerous and incompatible chemical processes in a single system. Such systems might create chemical 'factories' by, for example, feeding products from one compartment to serve as reactants in another. Generally such multi-compartment systems could orchestrate complex synthesis, catalysis or separation processes currently impossible or expensive and tedious to perform in vitro. Further, the use of synthetic lipid bilayer compartments in their work also suggests how synthetic smart vesicles containing circuitry might form channels connecting living and synthetic cells. Such hybrid systems might one day lead to new means of diagnosis or therapeutics. ❐

Yi Li and Rebecca Schulman*
Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, USA.
**e-mail: rschulm3@jhu.edu*

Published online: 14 December 2018
https://doi.org/10.1038/s41557-018-0192-7

References
1. Christian, J. L. *Wiley Interdiscip. Rev. Dev. Biol.* **1**, 3–15 (2011).
2. Grosberg, R. K. & Strathmann, R. R. *Annu. Rev. Ecol. Evol. Syst.* **38**, 621–654 (2007).
3. Mittelbrunn, M. & Sánchez-Madrid, F. *Nat. Rev. Mol. Cell Biol.* **13**, 328–335 (2012).
4. Elowitz, MichaelB. & Leibler, Stanislas *Nature* **403**, 335–338 (2000).
5. Basu, S. et al. *Nature* **434**, 1130–1134 (2005).
6. Buddingh', B. C. & van Hest, J. C. M. *Acc. Chem. Res.* **50**, 769–777 (2017).
7. Tayar, A. M., Karzbrun, E., Noireaux, V. & Bar-Ziv, R. H. *Nat. Phys.* **11**, 1037–1041 (2015).
8. Dupin, A. & Simmel, F. C. *Nat. Chem.* https://doi.org/10.1038/s41557-018-0174-9 (2018).
9. Eldar, A. & Elowitz, M. B. *Nature* **467**, 167–173 (2010).

NANOPARTICLE FUNCTIONALIZATION

Surface anchors target golden bullets

Gold nanomaterials are attractive for a variety of applications, including in medicine, but need to be made stable enough to operate in biological systems. Now, gold nanorods have been stabilized for photothermal therapy by sequential surface anchoring, using a bidendate PEG-based ligand that features a thiolate moiety and an Au–NHC moiety.

Guillaume Médard and Anthoula C. Papageorgiou

When gold gets small, things get interesting. Scientists have long been intrigued by the variety of colours obtained by controlling the structure of gold nanoparticles, which can now be made into different sizes and increasingly complex shapes. The colour of gold nanoparticles is determined by their surface plasmon resonance, that is, electronic oscillations (plasmons) at the surface of the nanoparticles that resonate with incident light. For gold, nanoscaling results in strong resonances in the visible regime. Beside the aesthetic appeal of these rainbow colours and their glass-staining applications, over the past decades the unique physicochemical properties of gold nanoparticles have found significant applications in fields ranging from catalysis to medicine. Shaped as nanorods, they have the potential to be actual golden bullets in laser-induced phototherapy — providing that they are stable enough to perform their therapeutic task. This stability has however been difficult to engineer. Writing in *Nature Chemistry*, an interdisciplinary team led by Jeremiah Johnson describes an exciting method to prepare nanorods with good lifetimes in biological environments[1].

Gold nanorods of approximately 40 nm length and 10 nm diameter have a strong surface plasmon resonance in the near infrared (NIR) region, and a weaker resonance in the visible region, owing to the electronic oscillation in the longitudinal and transverse directions, respectively. This leads to high adsorption in the NIR, which excites electrons. The subsequent de-excitation proceeds by a phonon release that transfers heat to the surroundings. Incidentally, light in the NIR region is able to penetrate the human body up to a few millimetres in depth. Additionally, gold nanorods accumulate in cancer tumours, owing to the tissues' dysregulated blood and lymph vascularization. Putting these parameters together, El-Sayed and co-workers pioneered[2] photothermal cancer therapy by NIR continuous laser excitation of gold-nanorods, which can selectively overheat the malign tissue to destruction (Fig. 1a). The heating cycles in the biological environment however take a toll on the gold nanorods: their shape shifts and with it the critical parameters for efficacious treatment are also lost[2].

The stabilization of gold at the nanoscale commonly comes courtesy of organic capping agents that can range from a surrounding polymer to smaller ionic or covalent ligands. The latter have certain advantages: they form relatively strong bonds with gold and their surface density may also be tuned to preserve access to the particle surface. Among these ligands, thiolates have attracted the most intense investigations. The Au–S interface has found a variety of applications in nanoparticle stabilization, in self-assembled monolayers and in single-